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Biogas, Biodiesel and Bioethanol as Multifunctional Renewable Fuels and Raw Materials

Venko Beschkov

Additional information is available at the end of the chapter

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Abstract

Nowadays the world economy is based mainly on petrol as an energy source and raw material for chemical products. The global economic growth in the past century has led to high energy consumption, mainly from fossil fuels, such as coal, oil, and natural gas. The extensive use of fossil fuels formed and stored underground for millions of years has made impossible for the present vegetation on Earth to treat the emitted carbon dioxide by photosynthesis, leading to strong emissions of carbon dioxide and greenhouse effect with the consequent climate changes. One of the ways to cope with this global problem is to close the carbon cycle in nature by the use of renewable biofuels enabling recycling the sources of biological origin by energy production and consumption of the resulting carbon by photosynthesis. Some of these biofuels are biogas (a mixture of methane and carbon dioxide) generated from organic waste; ethanol, produced by fermentation of carbohydrates; and biodiesel, produced by transesterification of lipids. Another feature of this approach is the utilization of organic waste as energy, thus leading to multiple benefits for the environment: waste treatment with energy production, closing the natural carbon cycle, and saving of fossil fuels. Biofuels with their feedstock also serve as raw materials for new technologies for chemicals being now produced from petrol, natural gas, and coal.

Keywords: biomass, renewable fuels, biogas, biodiesel, ethanol, carbon dioxide recycling

1. Introduction

During the twentieth century, the world economy was mainly based on petrol as an energy source and raw material for chemical products. Energy consumption has increased steadily

over the last century due to the world population growth and the technical progress development. The enormous economic growth on a global scale in the past century has led to the extensive use of fossil fuels, such as coal, oil, and natural gas.

A reason of concern was the extensive use of fossil fuels formed and stored underground for millions of years. It has made impossible for the present vegetation on Earth to treat the emitted carbon dioxide by photosynthesis. The result was greenhouse effect with the consequent climate changes. The climate changes assigned to the increased emissions of greenhouse gases forced humanity to develop alternative energy sources, one of them being biomass, either fresh or residual.

Another reason for humanity to turn to the renewable energy resources is the concern of depletion of the overall oil reserves [1], because the eight great economies (except Brazil) and many other nations depend on oil, the consequences of inadequate oil availability could be severe. Therefore, there are great incentives in exploring alternative energy sources.

One of the ways to cope with this global problem is to close the carbon cycle in nature by the use of renewable fuels enabling recycling the sources of biological origin by energy production and consumption of the resulting carbon by photosynthesis. Such biofuels are biogas (a mixture of methane and carbon dioxide) generated by anaerobic digestion of organic waste, ethanol, produced by fermentation of carbohydrates, and biodiesel, produced by transesterification of lipids.

The main feature of these approaches is the utilization of organic waste as energy, thus leading to multiple benefits for the environment: waste treatment with energy production, closing the natural carbon cycle, and saving of fossil fuels. Moreover, there are options to utilize the biofuels and their derivatives and residues as sources for chemical production.

2. Biogas

The anaerobic digestion of organic waste is a well spread process in nature. The huge amounts of natural gas collected underground are formed by this process during millions of years. The result is gas, containing about 95% methane with some contaminations. Nowadays, this process is used for agricultural waste treatment producing biogas with satisfactory heating capacity. Biogas is a mixture of methane and carbon dioxide with some contaminations of hydrogen sulfide, mercaptans, ethane, etc. The methane content varies from 55 to 90% volume depending on the substrate nature and content, the method of digestion, etc. The gas containing less than 50% methane is not combustible.

Biogas is broadly distributed in countries with developed agriculture (like India, China, Brazil, etc.), being a cheap and environmentally friendly option for the simultaneous solution of waste treatment problems and energy demand. Anaerobic digestion is also a convenient technology for activated sludge utilization and waste treatment in the food industry, pulp and paper industry, in household waste treatment, etc.

The anaerobic digestion with biogas production is a complicated process of consequent hydrolysis of organic macromolecules (carbohydrates and proteins) to oligosaccharides and peptides, acidogenesis to volatile fatty acids (mainly formic, acetic, and propionic), acetogenesis, and methanogenesis [2]. The overall process is shown in the scheme in **Figure 1**.

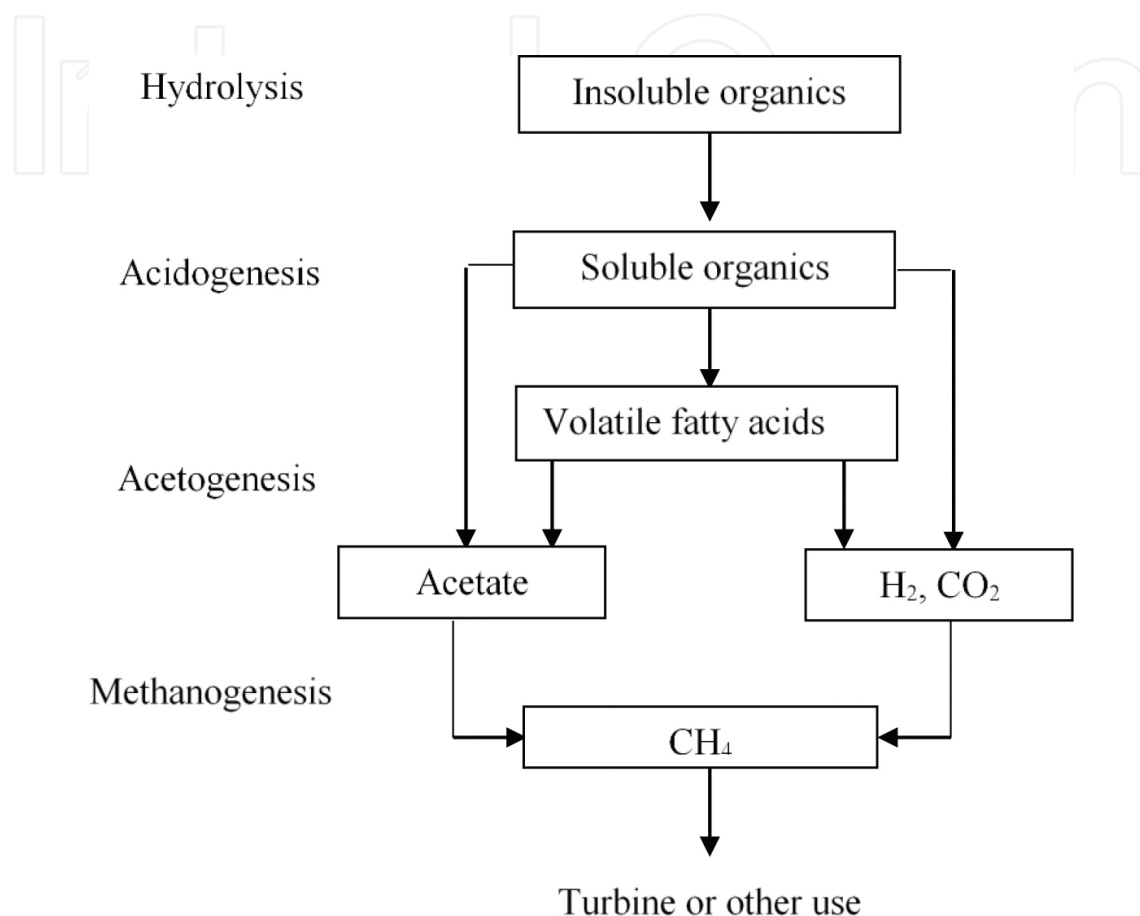
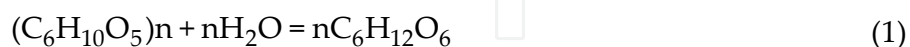


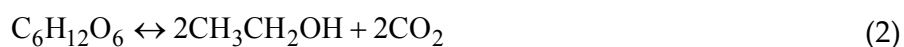
Figure 1. Four steps in biogas production [2].

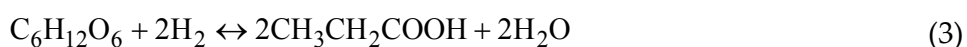
The gross chemical reaction describing the first step, i.e., hydrolysis is:



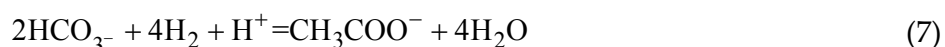
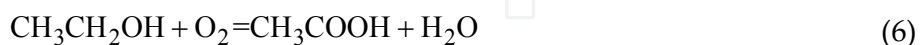
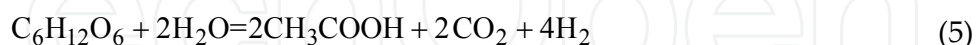
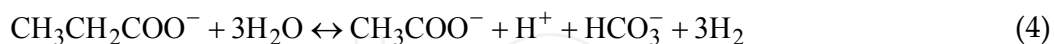
In the second step, acidogenic bacteria convert the products of hydrolysis into simple organic compounds, mostly short-chain carboxylic acids, ketones and alcohols.

The chemical reactions are shown below. Glucose is parallelly converted into ethanol and propionic acid:





The acetogenic reactions are



In the last step, methanogenic bacteria convert acetic acid into CH_4 and CO_2 :



There is another parallel pathway to produce methane by reduction of carbon dioxide resulting from formic acid degradation:



The activity of the digesting bacteria and biogas production of gas is most rapid in two temperature ranges: between 29°C and 41°C (fermentation is known as mesophilic) or between 49°C and 60°C (the thermophilic range). The mesophilic regime between 32°C and 35°C is more reliable for stable and continuous production of methane. Biogas produced outside this temperature range is rich of carbon dioxide, it is not combustible and that is why it has no calorific value. The thermophilic regime gives higher yield of biogas, but with less net energy efficiency because of the energy losses for high temperature maintenance.

Different methanogenic strains are responsible for these parallel and competitive processes. The bacteria from the genus *Methanosarcina* are capable to grow during the catabolism of acetate to CO_2 and CH_4 [3], whereas the strains *Methanobacterium* and *Methanobrevibacter* convert carbon dioxide by reduction of hydrogen to methane [4–8].

The balance between decarboxylation of acetic acid and carbon dioxide reduction is important for the methane content in the resulting biogas. If only decarboxylation of acetic acid occurs,

methane content will be 50% only. The high methane content in the biogas means that carbon dioxide reduction prevails.

Anyway, all methanogenic strain are vital in neutral media, i.e., for pH values between 6 and 8. Big deviations either in the acid domain or in the alkaline one lead to strong inhibition and even to death. Acidogenesis is one of the inevitable steps in biogas production. On one hand, methanogenesis is favorized by fatty acid formation, but on the other hand, it could be strongly inhibited by their accumulation due to the pH drop. In such cases, the produced gas is very rich in carbon dioxide and it is not combustible. That is why, one must be very careful in the feeding strategy by substrate and in the selection of bioreactor and flow organization.

One suitable way to minimize the effect of acid accumulation on the biogas formation is to distribute spatially the consecutive processes of biogas formation and to carry them out simultaneously. Such a construction is the baffled bioreactor separated into consecutive compartments fed from the one end with outflow at the other one, cf. **Figure 2**. It is known that such reactors are stable toward disturbances in feed, pH oscillations, temperature variations, etc. [9].

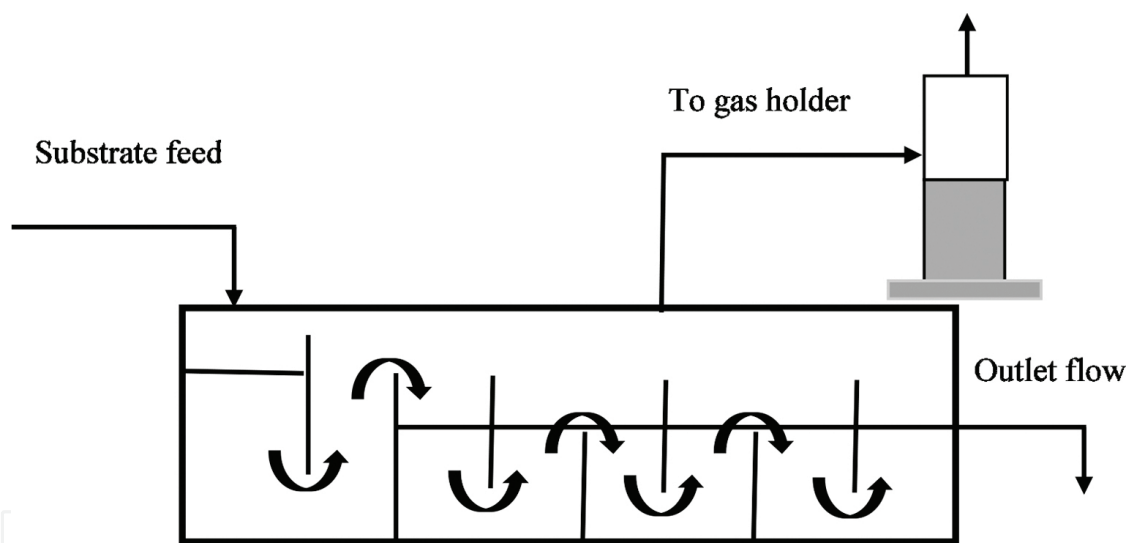


Figure 2. Multistage bioreactor for biogas production.

The main advantage of this type of reactor in the considered case is the distribution of the different consecutive processes (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) in different reactor compartments. Due to its one-way feeding, the intermediate products in one compartment passed as substrates to the next one. Because of this feeding organization, different bacteria are spontaneously cultivated, specialized to transform different intermediates of the overall methanogenesis.

There are successful applications of such bioreactor for biogas production by residual stillage from ethanol distillation as a feed [10]. The intermediate profile and the microbial distribution in eight compartment bioreactor for this process are illustrated in **Figure 3** by author's experimental data. Acetic and propionic acids prevail in the first three compartments where

reducing sugars are present, due to the hydrolysis of carbohydrates. Obviously, the first three steps of biogas production, i.e., hydrolysis, acidogenesis, and acetogenesis predominantly take place in the first three compartments where sugars are present and the concentration of methanogenic bacteria is very low or negligible. Methanogenics prevails in the next compartments 5–7, which corresponds to the very low acid concentration. There are few methanogenic bacteria in compartment 8 which corresponds to the negligible acid concentration. The biogas productivity rate for those experiments was ca. 4 vol.biogas/vol.reactor/day.

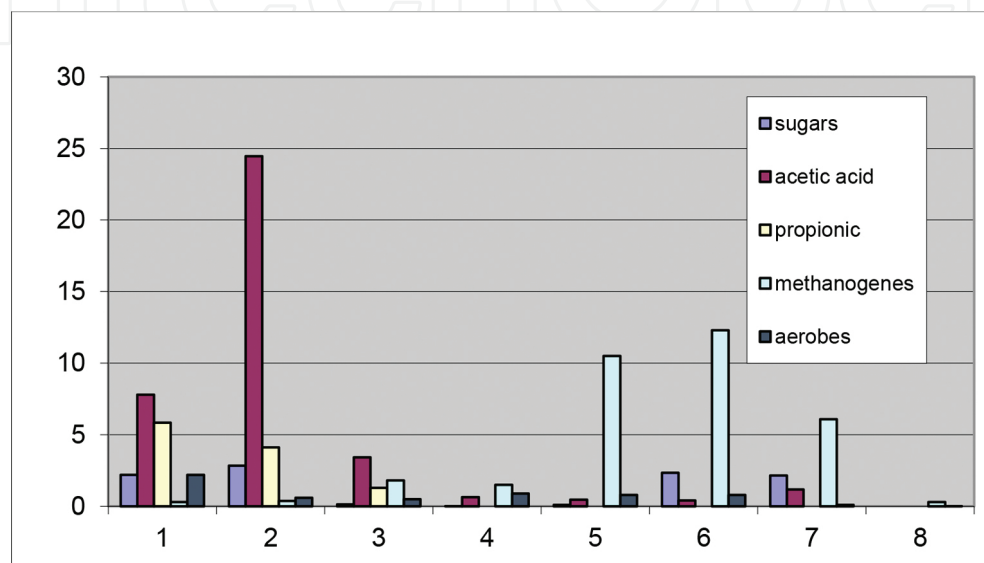


Figure 3. Acid and microbial profiles along the compartments in the bioreactor (own data).

The biogas produced by anaerobic digestion finds applications in different area. First, it could be used directly for heating purposes. Next, after some processing to remove carbon dioxide and sulfur-containing compounds the biogas could replace partially the natural gas for local applications. This biogas could be supplied directly in the pipelines, it could be used for the public transport and for electricity production by cogeneration.

Another promising application is the direct electricity production in fuel cells [11]. For this purpose, biogas should be scrubbed for carbon dioxide and sulfur compounds removal and then the purified methane could be fed to solid oxide fuel cells (SOFC) and molten carbonate fuel cells (MCFC). In this case, methane is directly converted to hydrogen and carbon monoxide by a catalyst in the anodic space. Another approach is to convert methane into carbon monoxide by steam reforming (SR) or partial oxidation reforming (POX) and consequent water-gas shift reaction to isolate hydrogen, supplied to a fuel cell:



2.1. Biogas as a source of organic fuels

There are some papers claiming to utilize biogas as a source for other organic fuel production by catalytic auto-thermal reforming [12, 13]. Another approach is to use biogas being a mixture of methane and carbon dioxide to produce synthesis gas (mixture of carbon monoxide and hydrogen) [14]:



Furthermore, the synthesis gas could be converted into light hydrocarbons by the Fischer-Tropsch process.

3. Biodiesel

Biodiesel consists of methyl or ethyl esters of fatty acids produced by transesterification of natural lipids. Different natural fats are used as raw materials, namely, rapeseed, soybean, processed residual sunflower oil, animal fats, and some kinds of algae. The latter are attractive because they can utilize the carbon dioxide from flue gases by photosynthesis thus reducing the emissions of greenhouse gases [15].

The energy content of biodiesel is within 37 and 40 MJ/L compared to 46 MJ/L of the traditional diesel fuel. Biodiesel does not contain sulfur compounds.

The idea for the use of vegetable oils as fuel for diesel engines is more than 100 years old [16, 17]. Just in the 1970s, the petrol crises and the enhanced environmental conscience in the modern societies have led to the secondary discovery of this possible alternative to the hydrocarbon-based fossil fuels. However, the direct use of vegetable oil as a fuel is not convenient, because of its very high viscosity, high flame point, trend to polymerization, etc., all leading to engine damage [18].

Transesterification with low alcohols is the best modification of natural oil for the biodiesel purposes.

Today, biodiesel is in commercial use throughout the world. It is used as a single fuel or blended with traditional diesel (with 30–36%).

Biodiesel is produced in the European Union since 1992. The world production attained 3.8 mln tons in 2005 to reach 3.7 mln tons only in the USA in 2007. The total world production for 2016 is about 15 mln tons.

3.1. Benefits of biodiesel use

Biodiesel does not contain sulfur and aromatic compounds and its use in the conventional engines leads to reduction of emissions of noncombusted hydrocarbons and carbon monoxide. Comparison of the emissions resulting by the use of biodiesel and traditional one is shown in **Table 1**.

Emission type	Biodiesel	Traditional diesel
	(% of EU standards from 1993)	
Carbon monoxide	60	180
Hydrocarbon total	90	150
Nitrogen oxides	65	60
Fine particulates	95	190

Table 1. Comparison of emissions released by biodiesel and conventional diesel fuel [19].

3.2. Problems in biodiesel production and use

The main disadvantage at biodiesel production and use is the uncertain standardization depending on the source of lipids. It reflects the different cetane number and the variable temperature of gelatinization depending on the esters and the raw material type.

Another severe problem is crude glycerol, released as byproduct after transesterification of lipids. Its amount is about 10% of the substrate and it is almost equal of the methanol used. This residual glycerol is contaminated by potassium hydroxide, water, some nonreacted lipids, some soaps, and monoglycerides and diglycerides. The low quality of this product makes it impossible for direct practical application.

Provided the annual world production of biodiesel is about 15 mln metric tons, one could expect that 1.5 mln metric tons of crude glycerol would be released. It is an enormous amount and it poses the necessity for its application and processing.

Pure glycerol has various practical applications but it could be hardly replaced by the residual crude glycerol after biodiesel production. That is why new application should be sought.

Recent studies show the opportunity for crude glycerol utilization as syngas by steam reforming [20–22], cf. Eq. (9). Other applications are proposed, for example, hydrogen production by photo-fermentation [23, 24], or as a fuel in fuel cells and microbial fuel cells [25, 26]. However, in these cases the contamination by methanol is not recommended [27, 28].

3.3. Glycerol utilization for hydrogen and other chemicals production

The large amounts of residual crude glycerol prompted to the search of simultaneous waste treatment and for new applications as a raw material, alternative to the petrol for the traditional organic synthesis [29–31]. Such efforts are directed toward production of chemicals of broad industrial importance, e.g., polyols as precursors of plastics (2, 3-butanediol, 1, 3-propandiol) [32–35], propionic acid [36, 37], succinic acid [38], or hydrocarbons by catalytic reforming [39], for epichlorohydrin, some ethers [40], polyesters, etc.

Among the potential applications of waste glycerol are the production of biodegradable polymers for packaging [31, 32, 41, 42], as antifreezing agents [43] as substrate for microbial syntheses, etc. Crude glycerol has been used as carbon source in the nutrition media for biopolymer production by the species *Bacillus* and *Pseudomonas* [44–46].

3.4. Microbial conversion of glycerol into chemical products

The bacterium *Rhodopseudomonas palustris* is capable for photo-fermentative conversion of crude glycerol to hydrogen [23]. The conversion rates and the yields depend on the concentrations of the added nitrogen containing compounds. Higher yields of hydrogen and also ethanol are registered at the use of *Enterobacter aerogenes* HU-101 [47]. There are works on the production of different chemicals from glycerol in microbial processes. Different bacteria (from the genera *Klebsiella*, *Clostridium*, and *Enterobacter*) are capable to convert glycerol, producing basic chemicals, differing by the intermediate reactions and products [30]. The metabolic pathway of glycerol conversion by bacteria from the genus *Klebsiella* was proposed and discussed by Saxena et al. [48] and Zhang et al. [49]. It is shown in **Figure 4**. It is seen that two diols (1,3-propanediol and 2,3-butanediol) are produced by two competitive mechanisms. Those two diols are interesting as precursors for polymer production, e.g., polypropylene and butadiene. Besides succinic and lactic acids are produced, ethanol too.

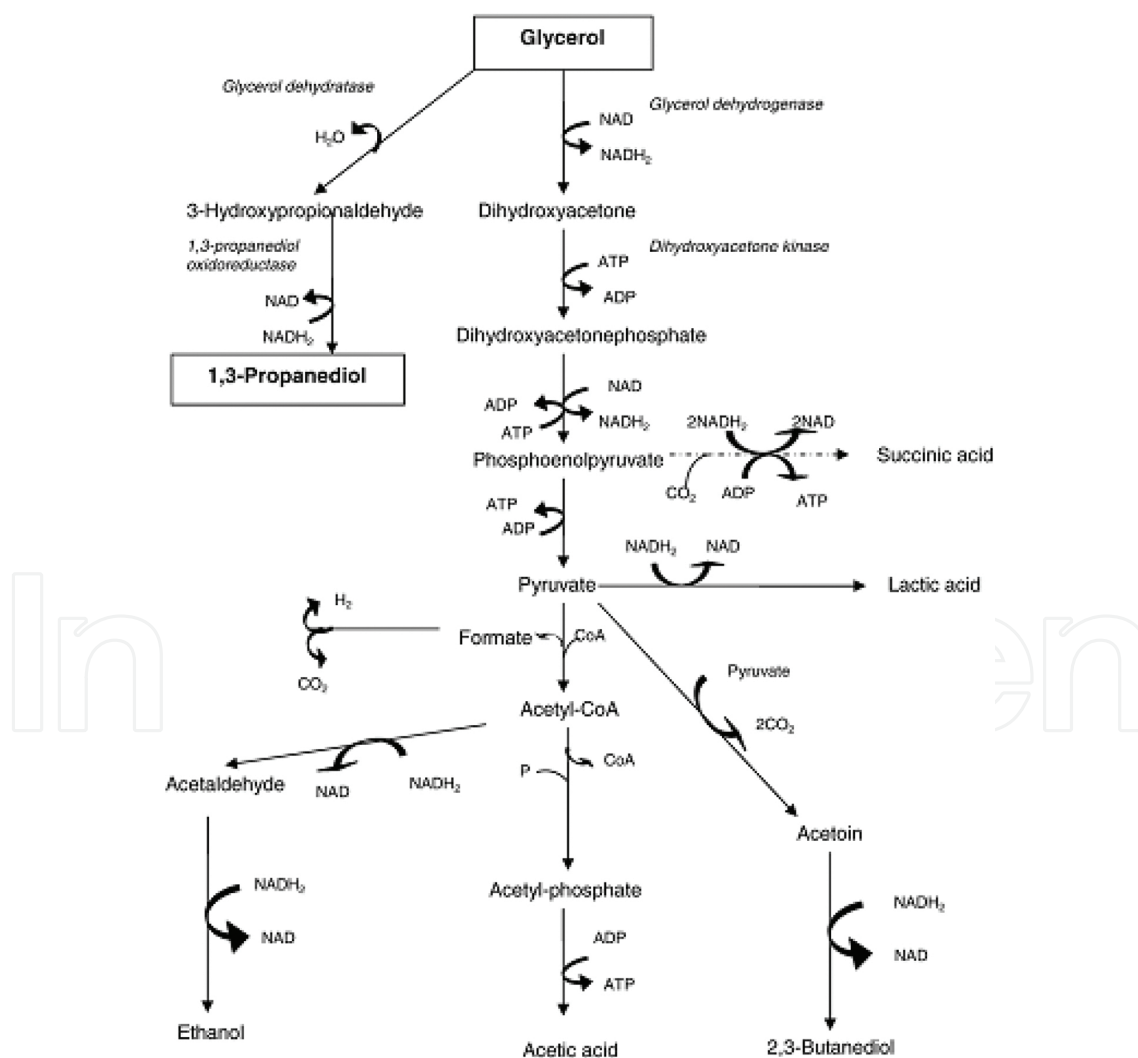


Figure 4. Metabolic pathway for glycerol digestion by bacteria from the genus *Klebsiella* [48, 49].

The studies of glycerol conversion at the metabolism of bacteria from the genus *Clostridium* show similar processes like in the previous case [50–52]. At *Clostridium* mainly 1,3-propanediol, organic acids (formic, acetic, butyric, and lactic) as well as n-butanol are produced.

Additionally, formic and acetic acids are also produced. These two carboxylic acids are very important for the consequent production of biogas being a mixture of methane and carbon dioxide.

The studies of the metabolism of *Enterobacter* bacteria show predominant formation of ethanol and hydrogen [53–55].

3.5. Glycerol for biogas production

The microbial production of acetic and formic acids from glycerol is interesting with the relationship of biogas production by anaerobic digestion. The two main pathways for biogas production by methanogenic bacteria are based on acetate decarboxylation, or carbon dioxide reduction by hydrogen, both produced from formic acid decomposition, cf. Eqs. (8) and (10).

Conversion of glycerol into biogas by anaerobic fermentation is an interesting option to produce renewable energy together with waste glycerol treatment [56–58]. It is reported that glycerol considerably enhances biogas formation by properly selected microbial population [59]. There are also many studies for the glycerol impact on biogas yield from various substrates, such as cattle dung [60–62], pig manure [57, 62, 63], activated sludge [64–66], as well as at more complicated mixtures of cellulose and household waste [57, 58, 67].

In any case, the results are considerable enhancement of biogas yield from 180 to 400% with respect to the reference substrate. It is typical, however, that the amounts of the added glycerol are restricted to 1–4% wt. from the main substrate. Addition of bigger amounts of glycerol leads to strong acidification of the broth and inhibition of methanogenesis [39, 56]. It means that no considerable amounts of crude glycerol could be utilized as biogas.

However, it was reported recently that crude glycerol may serve as a single substrate for biogas production with pretty high yield, i.e., 0.345 L biogas/g COD [68].

For attainment of maximum efficient biogas production different schemes of bioreactor feed are studied, as well as choice of the reactor construction, flow organization, etc.

4. Ethanol

Ethanol is a renewable energy source produced through fermentation of sugars. Ethanol is widely used as a partial gasoline replacement worldwide. Fuel ethanol that is produced from corn has been used in gasohol or oxygenated fuels since the 1980s. These gasoline fuels contain up to 10% ethanol by volume. As a result, the US transportation sector now consumes about 4540 million liters of ethanol annually, about 1% of the total consumption of gasoline. Recently, US automobile manufacturers have announced plans to produce significant numbers of flexible-fueled vehicles that can use an ethanol blend with 85% ethanol and 15% gasoline by

volume—alone or in combination with gasoline. Using ethanol-blended fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission.

Ethanol is also a safer alternative to methyl tertiary butyl ether (MTBE), the most common additive to gasoline used to provide cleaner combustion.

However, the cost of ethanol as an energy source is relatively high compared to fossil fuels. A dramatic increase in ethanol production using the current corn starch-based technology (or other cereals) may not be practical for small countries because corn production for ethanol will compete for the limited agricultural land needed for food and feed production. Additional drawback is the increasing prices of cereals used extensively as substrate for ethanol production by fermentation due to the enhanced demand and thus putting the third-world countries in disadvantaged position.

An alternative potential source for low-cost ethanol production is to utilize lignocellulosic biomass (LCB) such as straw, stems, cobs, grass, sawdust, wood chips, and forestry waste. This approach is known as “second-generation” ethanol production. Extensive research has been completed on conversion of lingo-cellulose to ethanol in the past two decades [69–74].

The conversion includes two main steps: pretreatment with hydrolysis of cellulose in the LCB to fermentable reducing sugars and fermentation of the sugars to ethanol [75].

The purpose of pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials. Physical, physical-chemical, chemical, and biological processes have been used for pretreatment of LCB. The hydrolysis is usually catalyzed by cellulolytic enzymes, and the fermentation is carried out by yeasts or bacteria. The factors that have been identified to affect the hydrolysis of cellulose include porosity (accessible surface area) of the waste materials, cellulose fiber crystallinity, and lignin and hemicellulose content [2, 75]. The presence of lignin and hemicellulose impedes the access of cellulases to cellulose, thus reducing the efficiency of the hydrolysis. Removal of lignin and hemicellulose, reduction of cellulose crystallinity, and increase of porosity in pretreatment processes can significantly improve the hydrolysis [76, 77].

Another disadvantage of the enzymatic hydrolysis of cellulose is the strong product inhibition of glucose and therefore the low-product concentrations and the low-process rate. The easier but not environmentally friendly way is to use acid hydrolysis by sulfuric acid [78]. In this case, higher concentrations of fermentable sugars and oligosaccharides are produced. The usual approach is to employ a two-step dilute acid hydrolysis, where the hemicellulose is hydrolyzed to xylose and recovered in the first stage and a more vigorous second-stage hydrolysis is employed for conversion of cellulose to glucose [79].

Several different organisms have been proposed for convert fermenting sugars into ethanol. The mostly spread ones are the yeast, *Saccharomyces cerevisiae*, due to its robust growth rate and high ethanol tolerance, up to 23% [80, 81]. The effort to use thermostable yeast has shown that they suffer from low ethanol tolerance [82].

The bacterium, *Zymomonas mobilis*, has been shown to produce higher ethanol yields but with lower ethanol tolerance [82].

After fermentation ends the “beer” containing 2–12% ethanol is subjected to distillation to produce the azeotropic mixture of 96% (vol.) ethanol and 4% water. This mixture is not appropriate for blending with gasoline for the water separation, particularly at low temperatures. That is why additional drying is required to reach water content of less than 1%.

The classical methods are extractive distillation by adding solvents like benzene, cyclohexane, or ether to break the azeotrope.

Most advantageous is the molecular sieve drying technology, where the azeotrope is passed through a bed of synthetic zeolite with uniform pore sizes which preferentially adsorb water molecules. After the bed becomes saturated, it is regenerated by heating or evacuating the bed to remove the adsorbed water. The most efficient technology is the vapor-phase “pressure swing” adsorption molecular sieve process [83]. Nowadays, this process is preferred to the classical extractive distillation due to the clean process and the lack of side chemical products due to extraction and distillation.

The problem to be solved is the stillage processing. Stillage is the waste after ethanol distillation and it contains a lot of cellulose residues, nonfermented oligosaccharides, proteins, etc., with COD reaching 70 kg/m³. The stillage amounts are between 1 L/kg feedstock for cereals and 20 L/kg for cellulosic substrate from coniferous origin.

There are different ways to treat this waste. One of them is to use it as animal feed after evaporation and concentration. Another option is to use it as substrate for single cell protein production with the subsequent use as animal feed of the residue. The simplest and the straightforward method is to use stillage for biogas production by anaerobic digestion [84, 85]. According to our experience, the produced biogas has over 70% (vol.) methane content. The COD was decreased from 70 to 1 g/L (over 98% efficiency) and after some additional treatment the wastewater could safely be discharged or used for irrigation.

There is a relatively new proposal for consolidated bioprocessing (CBP) of lingo-cellulosic materials consisting in cellulase production, substrate hydrolysis, and fermentation accomplished in a single process by cellulolytic microbes [86].

CBP offers the potential for lower biofuel production costs due to simpler substrate processing, low energy inputs, and higher conversion efficiencies compared to separate hydrolysis and fermentation processes. It is an economically attractive goal for “third-generation” biofuel production.

4.1. Ethanol applications as fuel and raw materials

The use of ethanol as fuel depends on the oil prices on the global market and the local regulations in the different states. However, there are other options to use the “bioethanol” as a substrate and a raw material for chemical purposes. Besides the well-known applications as commodity product, chemical reactant, and solvent, ethanol may serve as a source of hydrogen production by steam reforming [87, 88] or chemical products, like ethylacetate [89, 90].

4.2. Problems and drawbacks

The main problems associated to ethanol production for fuel purposes are either from economical or environmental point of view. The economic problems are related to the prices of cereals competing its application as food. Therefore, the extensive use of cereals for industrial or fuel purposes may be unfavorable for their alimental needs. Next, the demand of new area for crop growing may lead to deforestation and disturbing the biodiversity and environmental balance.

The use of second-generation raw materials for ethanol production (cellulose-based waste) is restricted due to environmental reasons. Not all of the waste lingo-cellulosic biomass could be safely converted into ethanol without disturbing the natural ecological processes. The extensive use of lingo-cellulose for ethanol production could lead to deforestation and threat on biodiversity in large area of land. That is why decision making on the size and the rate of lingo-cellulosic waste use for this and for any other purpose should be very carefully, after precise and thorough environmental analysis.

5. Carbon dioxide utilization

Unfortunately, each of the described processes of biomass utilization ends with the inevitable release of carbon dioxide, resulting of the fuel combustion. Next, crops growing in industrial scale require considerable input of energy, most frequently taken from oil-based fuels. That is why abiotic carbon dioxide utilization and conversion is a major task for the future research and technology of biomass-based renewable fuels. There are two trends for this challenge: utilization photosynthesis by vegetation and recycling the biomass by chemical or electro-chemical reduction to organic fuels, like methane.

The first one is to pass flue gases containing carbon dioxide through greenhouse area containing algae capable to produce lipids and other organics being biofuels. Algae can be grown in open ponds, closed-loop systems, and photo-bioreactors. Algae are capable of much higher yields with lower resource inputs than other feedstock and that is why they are moved to an own category. The following biofuels could be produced by algae: biodiesel, butanol, gasoline, methane, ethanol, and kerosene [91, 92].

There are some problems associated with the efficiency of photosynthesis for industrial purposes, the utilization rate of carbon dioxide, etc. Another drawback regarding algae is that biofuel produced from them are less chemically stable than biodiesel produced from other sources because the biofuels have unsaturated bonds in their molecules and they are subjected to spontaneous polymerization.

Another approach is to utilize carbon dioxide as a raw material for various chemical products, like ethers, dimethylcarbonate, as antiknocking additive, monomers for plastics production, acyl carbonates [93]. All of these products are currently produced from petrol and that is why carbon dioxide recycling is important to greenhouse emissions but also to the reduced use of oil as a whole.

On the other hand, carbon dioxide is irreplaceable tool for supercritical extraction of biologically active and thermo-unstable substances from natural products.

An attractive approach is to reduce electrochemically carbon dioxide to methane in presence of methanogenic bacteria [94–97] or to ethanol and acetic acid [98].

6. Conclusions

The present review demonstrates different options for biomass utilization to replace, at least partially, the use of fossil fuels and thus to reduce the pressure of greenhouse gases emissions and to close the carbon cycle in nature within the present times. The biomass and the waste of biofuel production could be also utilized as raw material for various chemical manufacturing currently produced from oil. However, in some cases this option may additionally influence the environment and create secondary pollution. It could happen in cases of bioethanol and biodiesel production when large area of land is required for crop growth and much energy for crop production is requested. That is why local solutions about the use of renewable fuels based on biomass should be made very careful after thorough environmental analysis.

More attractive option is biogas production and utilization, because it is always related to simultaneous waste processing and energy production with closing the carbon cycle at local level.

Of course, the most promising research is dedicated to carbon dioxide recycling turning it to fuels or value-added chemicals.

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Author details

Venko Beschkov

Address all correspondence to: vbeschkov@yahoo.com

Institute of Chemical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

References

- [1] Campbell, C. J. and Laherrere, H. 1998, The end of cheap oil, *Scientific American*, 3, 78–83.

- [2] Garcia-Heras, J. L. Reactor sizing, process kinetics, and modelling of anaerobic digestion of complex wastes, In: J. Mata-Alvarez (editor), Biomethanization of the organic fractions in municipal solid wastes, IWA-Publishing, London, UK, 2003.
- [3] Ferry, J. G. 1997, Enzymology of the fermentation of acetate to methane by *Methanosarcina thermophila*., Biofactors, 6, 25–35.
- [4] Conrad R. 2005, Quantification of Methanogenic Pathways Using Stable Carbon Isotopic Signatures: A Review and a Proposal., Organic Geochemistry, 36, 739–752.
- [5] Zyakun, A.M., Bondar, V.A., Laurinavichus, K.S., Shipin, S.S., Belyaev, S.S. and Ivanov, M.V. 1988, Fractionation of carbon isotopes under the growth of methane-10 producing bacteria on various substrates (in Russian), Mikrobiologicheskii. Zhurnal, 50, 16–22.
- [6] Gelwicks, J. T., Risatti, J. B. and Hayes, J. M. 1994, Carbon isotope effects associated with acetoclastic methanogenesis, Applied and Environmental Microbiology, 60, 467–472.
- [7] Games, L. M., Hayes, J. M. and Gunsalus, R. P. 1978, Methane-producing bacteria: Natural fractionations of the stable carbon isotopes, Geochimica and Cosmochimica Acta, 42, 1295–1297.
- [8] Krzycki J.A., Kenealy W.R., deNiro M.J. , Zeikus J.G., Stable Carbon Isotope Fractionation by *Methanosarcina barkeri* during Methanogenesis from Acetate, Methanol, or Carbon Dioxide-Hydrogen. 1987, Applied and Environmental Microbiology, 53, 2597–2599.
- [9] Grobicki, A., Stuckey, D.C. 1992, Hydrodynamic characteristics of the anaerobic baffled reactor, Water Research, 26, 371–378.
- [10] Wilkie, A. C., Riedesel, K. J. and Owens, J. 2000, Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks, Biomass and Bioenergy 19, 63–102.
- [11] O'Hayre, R., Cha, S. W., Collela, W., Prinz, F. B. Fuel Cell Fundamentals (2nd Edition), John Wiley & Sons, N. Jersey, 2009, pp. 371–395.
- [12] Cassinelli, W.H., Damyanova, S., Parizotto, N.V., Zanchet, D., Bueno, J.M.C. and Marques, C.M.P. 2014, Applied Catalysis A: General, 475, 256–269.
- [13] Mortola, V.B., Damyanova, S., Zanchet, D., and Bueno, J.M.C. 2011, Surface and structural features of Pt/CeO₂-La₂O₃-Al₂O₃ catalysts for partial oxidation and steam reforming of methane, Applied Catalysis B Environmental 107(3–4), 221–236.
- [14] Damyanova S., Pawelec, B., Arishtirova, K. and Fierro, J.L.G. 2011, Biogas reforming over bimetallic PdNi catalysts supported on phosphorus-modified alumina, Fuel and Energy Abstracts 36(17),10635–10647.
- [15] Clarens, A.F., Resurreccion, E.P., White, M.A. and Colosi, L.M. 2010, Environmental life cycle comparison of algae to other bioenergy feedstocks, Environmental Science Technology, 44, 1813–1819.

- [16] Knothe, G. 2001, Historical perspectives on vegetable oil – based diesel fuels. *Inform* 12 (11), 1103-1107. http://biodiesel.org/reports/20011101_gen-346.pdf.
- [17] Henriques, R. 1898, Über partielle Verseifung von Ölen und Fetten. *Zeitschrift für Angewandte Chemie*, 15, 338–345.
- [18] Srivastava, A. and R. Prassad. 2000, Triglycerides-based diesel fuels. *Renewable and Sustainable Energy Reviews*, 4, 111–133.
- [19] van Dyne, D.L. and Raymer, P.L. 1992, Biodiesel production potential from industrial rapeseed in the southeastern U.S., Report for The Southeastern Regional Biomass Energy Program, pp. 1–20. http://biodiesel.org/reports/19921031_gen-239.pdf.
- [20] Dou, B., Dupont, V. and Williams, P. T. 2008, Computational fluid dynamics simulation of gas – solid flow during steam reforming of glycerol in a fluidized bed reactor. *Energy Fuels*, 22, 4102–4108.
- [21] Adhikari, S., Fernando, S. D., To, S. D. F., Bricka, R. M., Steele, P. H. and Haryanto, A. 2008, Conversion of glycerol to hydrogen via a steam reforming process over Nickel catalysis. *Energy Fuels*, 22, 1220–1226.
- [22] Avasthi, K.S. R.N. Reddy, R.N., Patel, S. 2013, Challenges in the Production of Hydrogen from Glycerol – A Biodiesel Byproduct Via Steam Reforming Process, *Procedia Engineering*, 51, 423–429.
- [23] Sabourin-Provost, G. and Hallenbeck, P. C. 2009, High yield conversion of a crude glycerol fraction from biodiesel production to hydrogen by photofermentation. *Bioresource Technology*, 100, 3513–3517.
- [24] S. Adhikari, S. D. Fernando, A. Haryanto, 2009, Hydrogen production from glycerol: An update, *Energy Conversion and Management*, 50 (10), 2600–2604.
- [25] Ragsdale, S. R. and Ashfield, C. B. 2008, Direct–glycerin fuel cell for mobile applications. *ECS Transactions*, 16, 1847–1854.
- [26] Arechederra, R. L. and Minter, S. D. 2009, Complete oxidation of glycerol in an enzymatic biofuel cell. *Fuel Cells*, 9, 63–69.
- [27] Lammers, P.J., Kerr, B.J., Weber, T.E., Bregendahl, K., Lonergan, S. M., Prusa, K. J., Ahn, D. U., Stoffregen, W. C., Dozier, W. A. and Honeyman, M. S. 2008, Growth performance, carcass characteristics, meat quality and tissue histology of growing pigs fed crude glycerin-supplemented diets. *Journal of Animal Science*, 86, 2962–2970.
- [28] Yang, F. X., Hanna, M. A. and Sun, R. C. 2012, Value-added uses for crude glycerol - A byproduct of biodiesel production. *Biotechnology and Biofuels*, 5, 5–13.
- [29] Pagliaro, M. and Michele Rossi, M. *The Future of Glycerol: New usages for a versatile raw material*, RSC Publishing, Cambridge. ISBN: 0854041249, pp 32–81.
- [30] Da Silva, G. P., Mack, M. and Contiero, J. 2009, Glycerol: A promising and abundant carbon source for industrial microbiology, *Biotechnology Advances*, 27, 30–39.

- [31] Ashby, R. D., Wyatt, V. T., Foglia, T. A. and Solaiman, D. K. Y. Industrial products from biodiesel glycerol, in: Biocatalysis & Bioenergy, Hou, C. T. and Shaw, J. J. (Eds.), John Wiley & Sons, Inc., Hoboken, 2009, pp. 131–154.
- [32] Biebl H., Menzel K., Zeng A.P. and Deckwer W.D., 1999, Microbial production of 1,3-propanediol, Applied Microbiology and Biotechnology, 52, 289–297.
- [33] Wilkens, E., Ringel, A.K., Hortig, D., Willke, T. and Vorlop, K.D. 2012, High-level production of 1, 3-propanediol from crude glycerol by *Clostridium butyricum* AKR102a, Applied Microbiology and Biotechnology, 93,1057–1063.
- [34] Oh, B. R., Seo, J. W., Heo, S. Y., Hong, W. Y., Luo, L.H., Son, J. H., Park, D. H. and Kim, C. H. 2012, Bioprocess and Biosystems Engineering, 35, 159–165.
- [35] Fermentive production of 1,3-propanediol; United States Patent 5254467; <http://www.freepatentsonline.com/5254467.html>.
- [36] Himmi, E. H., Bories, A., Boussaid, A., Hassani, L. 2000, Propionic acid fermentation of glycerol and glucose by *Propionibacterium acidipropionici* and *Propionibacterium freudenreichii* ssp. *Shermanii*, Applied Microbiology and Biotechnology, 53, 435–440.
- [37] Boyaval, P. and Corre, C. 1995, Production of propionic acid, Lait, 75, 453–461.
- [38] Lee, P.C., Lee, W.G., Lee, S.Y., Chang, H.N., 2001, Succinic acid production with reduced by-product formation in the fermentation of *Anaerobiospirillum succiniciproducens* using glycerol as a carbon source, Biotechnology and Bioengineering, 72, 41–48.
- [39] López, J.Á.S., De los Ángeles Martín Santos, M., Pérez, A.F.C. and Martín, A.M. 2009, Bioresource Technology, 100, 5609–5615.
- [40] Klepáčová, K., Mravec, D., Hájeková, E. and Bajus, M. 2003, Petroleum and Coal, 45(1-2), 54–57.
- [41] Brauneegg, G., Genser, K., Bona, R., Haage, G., Schellauf, F., Winkler, E. 1999, Production of PHAs from agricultural waste material. Macromolecular Symposia, 144, 375–383.
- [42] Tohoku Electric turns glycerin into bioplastics, <http://www.greencarcongress.com/2007/08/tohoku-elec-dev.html>
- [43] Todorova, S. Utilization of waste glycerol from biodiesel production by *Bacillus subtilis*, science & technologies, nautical & environmental studies, Patent BG 110823: Liquid Substance for Defreezing of Roads and Highways, 2, 2015, pp. 52–56.
- [44] Sathianachiyar, S. and Devaraj, A. 2013, Biopolymer producing by bacterial species using glycerol, a byproduct of biodisel. International Journal of Scientific and Research Publications, 3, 8, 1–5.
- [45] Saimmai, A., Rukadee, O., Sbhon, V. and Maneerat, S. 2012, Biosurfactant production by *Bacillus subtilis* TD4 and *Pseudomonas aeruginosa* SU7 grown on crude glycerol

obtained from biodiesel production plant as sole carbon source. *Journal of Scientific and Industrial Research (India)*, 71, 06, 396–406.

- [46] Anastacio, G.S., Santos, K. O., Suarez, P. A., Torres, F. A., De Marco, J. L. and Parachin, N. S. 2014, Utilization of glycerin byproduct derived from soybean oil biodiesel as a carbon source for heterologous protein production in *pichia pastoris*. *Bioresource Technology*, 152, 505–510.
- [47] Ito, T., Nakashimada, Y., Senba, K., Matsui, T. and Nishio, N. 2005, Hydrogen and ethanol production from glycerol-containing wastes discharged after biodiesel manufacturing process. *Journal of Bioscience and Bioengineering*, 100, 260–265.
- [48] Saxena, R., Anand, P., Saran, S. and Isar, J. 2009, *Biotechnology Advances*, 27, 895.
- [49] Zhang, Y., Huang, Z., Dua, C., Li, Y. and Cao, Z. 2009, *Metabolic Engineering*, 11, 101–106.
- [50] González-Pajuelo, M., Meynial-Salles, I., Mendes, F., Soucaille, P. and Vasconcelos, I. 2006, *Applied Environmental Microbiology*, 72(1), 96–101.
- [51] Biebl H. 2001, Fermentation of glycerol by *Clostridium pasteurianum*-batch and continuous culture studies, *Journal of Industrial Microbiology and Biotechnology*, 27(1), 18–26.
- [52] Szymanowska-Powalowska, D. 2014, 1,3-Propanediol production from crude glycerol by *Clostridium butyricum* DSP1 in repeated batch, *Electronic Journal of Biotechnology*, 17 (6), 322–328.
- [53] Reungsang, S., Sittijunda, Angelidaki, I. 2013, Simultaneous production of hydrogen and ethanol from waste glycerol by *Enterobacter aerogenes* KKU-S1, *International Journal of Hydrogen Energy*, 38(4), 1813–1825.
- [54] Raymond E., Nwachukwu, S., Shahbazi, A., Wang, L., Worku, M., Ibrahim, S. and Schimmel, K. 2013, Optimization of cultural conditions for conversion of glycerol to ethanol by *Enterobacter aerogenes* S012, *AMB Express*, 3, 12. doi: 10.1186/2191-0855-3-12
- [55] Jitrwung, R. and Yargeau, V. 2015, Biohydrogen and bioethanol production from biodiesel-based glycerol by *Enterobacter aerogenes* in a continuous stirred tank reactor, *International Journal of Molecular Sciences*, 16, 10650–10664. doi:10.3390/ijms160510650
- [56] Kolesárová, N., Hutňan, M., Špalková, V., Lazor, M. (2010). In: *Proceedings from the 37th International Conference of SSCHE, Tatranske' Matliare, Slovakia*, 1126–1139.
- [57] Yang, Y., Tsukahara, K. and Sawayama, S. 2008, Biodegradation and methane production from glycerol-containing synthetic wastes with fixed-bed bioreactor under mesophilic and thermophilic anaerobic conditions. *Process Biochemistry*, 43(4), 362–7.
- [58] Amon, T., Kryvoruchko, V., Amon, B. and Schreiner, M. Investigations on the effect of crude glycerol from biodiesel production as addition enhancing the biogas generation

from silomais, grain mare, rapeseed cake and pig manure (in German). Institut für Landtechnik, Universität für Bodenkultur Wien. Source: http://www.nas.boku.ac.at/fileadmin/_/H93/H931/AmonPublikationen/SEEGEndbericht.pdf; 2004 [accessed 25.05.09].

- [59] Glycerin as a biogas feedstock. <http://news.mongabay.com/bioenergy/2006/12/glycerin-as-biogas-feedstock.html>
- [60] Castrillón, L., Fernández-Nava, Y., Ormaechea, P. and Marañón, E. 2001, Optimization of biogas production from cattle manure by pre-treatment with ultrasound and co-digestion with crude glycerin, *Bioresource Technology*, 102(17), 7845–7849.
- [61] Robra, S., Serpa da Cruz, R., Oliveira, A.M., Almeida Neto, J.A. and Santos J.V. 2010, Generation of biogas using crude glycerin from biodiesel production as a supplement to cattle slurry. *Biomass and Bioenergy*, 34(9), 804–806.
- [62] Astals, S., Nolla-Ardèvol, V., Mata-Alvarez, J. 2012, Anaerobic co-digestion of pig manure and crude glycerol at mesophilic conditions: Biogas and digestate, *Bioresource Technology*, 110, 63–70.
- [63] Wohlgemut, O. 2009, Co-Digestion of Hog Manure with Glycerol to Boost Biogas and Methane Production, MSc. Thesis, University of Manitoba, Winnipeg, Manitoba, p. 51.
- [64] Siles, J.A., Martín, M.A., Chica, A.F. and Martín, A. 2010, Anaerobic co-digestion of glycerol and wastewater derived from biodiesel manufacturing. *Bioresource Technology*, 101(16), 6315e21.
- [65] Fountoulakis, M.S., Petousi, I, Manios, T. 2010, Co-digestion of sewage sludge with glycerol to boost biogas production. *Waste Management*, 30(10), 1849e53.
- [66] Fountoulakis, M.S. and Manios, T. 2009, Enhanced methane and hydrogen production from municipal solid waste and agro-industrial by-products co-digested with crude glycerol, *Bioresource Technology*, 100(12), 3043–3047.
- [67] Ostrem, K. Greening waste: Anaerobic Digestion for Treating the Organic Fraction of Municipal Solid Wastes, M.S. Thesis, Columbia University, May 2004, p. 6.
- [68] Hutnan, M., Kolesárová, N. and Bodik, I. 2013, Anaerobic digestion of crude glycerol as sole substrate in mixed reactor. *Environmental Technology*, 34(13–16), 2179–87.
- [69] Sassner, P., Galbe, M. and Zacchi, G. 2008, Techno-economic evaluation of bioethanol production from three different lignocellulosic materials, *Biomass and Bioenergy*, 32(5), 422–430.
- [70] Schneider, H., Maleszka, R., Neirinck, L., Veliky, I. A., Wang, P. Y. and Chan, Y. K. 1983, Ethanol production from D-xylose and several other carbohydrates by *Pachysolen tannophilus* and other yeasts, *Pentoses and Lignin*, 27, 57–71.
- [71] Shafizadeh, F. and Lai, Y.Z. 1975, Thermal degradation of 2-deoxy-D-arabino-hexonic acid and 3-deoxy-D-ribo-hexono-1,4-lactone, *Carbohydrate Research*, 42(1), 39–53.

- [72] Singh, A., Pant, D., Korres, N. E., Nizami, A.-S., Prasad, S. and Murphy, J. D. 2010, Key issues in life cycle assessment of ethanol production from lignocellulosic biomass: Challenges and perspectives, *Bioresource Technology*, 101(13), 5003–5012.
- [73] Velásquez-Arredondo, H. I., Ruiz-Colorado, A. A. and De Oliveira, S. 2010, Ethanol production process from banana fruit and its lignocellulosic residues: Energy analysis, *Energy*, 35(7), 3081–3087.
- [74] Sukumaran, R. K., Surender, V. J., Sindhu, R., Binod, P., Janu, K. U., Sajna, K. V., Rajasree, K. P. and Pandey, A. 2010, Lignocellulosic ethanol in India: Prospects, challenges and feedstock availability, *Bioresource Technology*, 101(13), 4826–4833.
- [75] Yang, B. and Wyman, C. E. 2008, Pretreatment: The key to unlocking low-cost cellulosic ethanol, *Biofuels, Bioproducts, and Biorefining*, 2, 26–40 .
- [76] Kerr, R. A. 2005, Bumpy road ahead for world's oil. *Science*, 310, 1106–1108.
- [77] Tassinari, T., Macy, C. and Spano, L. 1980, Energy-requirements and process design considerations in compression-milling pretreatment of cellulosic wastes for enzymatic-hydrolysis. *Biotechnology and Bioengineering Symposium*, 22, 1689–1705.
- [78] Mackie, K., Deverell, K. and Callander, I. Aspects of wood hydrolysis via the dilute sulphuric acid process. In: Duckworth HE, Thompson EA, editors. *International Symposium on Ethanol from Biomass*, 1982 Oct 13-15; Winnipeg, Canada. Ottawa: The Royal Society of Canada, 1983. pp. 271–308.
- [79] Beck, M. J. and Strickland, R. C. 1984, Production of ethanol by bio-conversion of wood sugars derived from two-stage dilute acid hydrolysis of hardwood. *Biomass*, 6, 101–110.
- [80] Ingledew, W. M. The biochemistry of alcohol production In: Lyons TP, Kelsall DR, Murtagh JE, editors. *The Alcohol Textbook*. Nottingham, UK: Nottingham University Press, 1995. pp. 55–79.
- [81] Lynd, L. R., Ahn, H. J., Anderson, G., Hill, P., Kersey, D. S. and Klapatch, T. 1991, Thermophilic ethanol production: investigation of ethanol yield and tolerance in continuous culture. *Applied Biochemistry and Biotechnology*, 28/29, 549–570.
- [82] Esser, K., Karsch, T. 1984, Bacterial ethanol production: Advantages and disadvantages. *Process Biochemistry*, 19(3), 116–121.
- [83] Swain, R. L. B. Molecular sieve dehydrators. How they became the industry standard and how they work. In: Jacques KA, Lyons TP, Kelsall DR, editors. *The Alcohol Textbook*, 3rd ed. Nottingham, UK: Nottingham University Press, 1999. pp. 289–293.
- [84] Kinoshita, C. M. 1991, Cogeneration in the Hawaiian sugar industry. *Bioresource Technology*, 35, 231–237.
- [85] Perea, P. 1981, The merits of excess bagasse as fuel for generating electricity. *Sugar y Azucar*, 76(5), 42–46.

- [86] Carere, C. R., Sparling, R. Cicek, R and Levin, D. B. 2008, Third Generation Biofuels via Direct Cellulose Fermentation, *International Journal of Molecular Sciences*, 9(7), 1342–1360.
- [87] Andonova, S., Naves de Ávila Neto, C., Arishtirova, K. and Damyanova, S. 2011, Structure and redox properties of Co promoted Ni/Al₂O₃ catalysts for oxidative steam reforming of ethanol, *Applied Catalysis B Environmental*, 105(3), 346–360.
- [88] Zanchet, D., Santos, J. B. O., Damyanova, S., Gallo, J. M. R. and Bueno, J. M. C. 2015, Toward Understanding Metal-Catalyzed Ethanol Reforming, *ACS Catalysis*, 5, 3841–3863.
- [89] Sato, A. G., Volanti, D. P., Meira, D. M., Damyanova, S., Longo, E. and Bueno, J. M. C. 2013, Effect of the ZrO₂ phase on the structure and behavior of supported Cu catalysts for ethanol conversion, *Journal of Catalysis*, 307, 1–17.
- [90] Freitas, I. C., Damyanova, S., Oliveira, D.C., Marques, C. M. P. and Bueno, J. M. C. 2014, Effect of Cu content on the surface and catalytic properties of Cu/ZrO₂ catalyst for ethanol dehydrogenation, *Journal of Molecular Catalysis A Chemical*, 381, 26–37.
- [91] Optimizing biofuel production from algae using carbon dioxide emissions, February 24, 2016, American Chemical Society, <https://www.sciencedaily.com/releases/2016/02/160224151017.htm>
- [92] Demirbas, A. 2011, Biodiesel from algae, biofixation of carbon dioxide by microalgae: A solution to pollution problems, *Applied Energy*, 88, 3541–3547.
- [93] Sankaranarayanan, S., Srivasan, K. 2012, Carbon dioxide – A potential raw material for the production of fuel, fuel additives and bio-derived chemicals, *Indian Journal of Chemistry*, 51A, 1252–1262.
- [94] Hara, M., Onaka, Y., Kobayashi, H., Fu, Q., Kawaguchi, H., Vilcaez, J. and Sato, K. 2013, Mechanism of electromethanogenic reduction of CO₂ by a thermophilic methanogen, *Energy Procedia*, 37, 7021–7028.
- [95] Kuhl, K. P., Hatsukade, T., Cave, E. R., D. N., Kibsgaard, J. and Jaramillo, T. F. 2014, Electrocatalytic conversion of carbon dioxide to methane and methanol on transition metal surfaces, *Journal of the American Chemical Society*, 136(40), 14107–14113.
- [96] Zhu, Y., S., Ye, Y., Zhang, X., Wang, L., Zhu, W., Cheng, F. and Tao, F. 2012, Catalytic conversion of carbon dioxide to Methane on Ruthenium–Cobalt Bimetallic Nanocatalysts and Correlation between Surface Chemistry of Catalysts under Reaction Conditions and Catalytic Performances, *ACS Catalysis*, 2(11), 2403–2408.
- [97] Cheng, S. and Logan, B. E. Electromethanogenic reactor and processes for methane generation. US Patent No. 8,440,438, issued May 14, 2013.
- [98] Min, S. U., Yong, J., Yao, Z., Ping G. and Daping, L. 2013, Coupled bioelectrochemical system for reducing CO₂ to simple organic compounds in the presence of H₂, *Chinese Journal of Applied Environmental Biology*, 19(5), 827–832.

